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(54) Title: HLA BINDING PEPTIDES AND THEIR USES

(57) Abstract

The present invention provides peptide compositions capable of specifically binding selected MHC alleles and inducing T cell activation in T cells restricted by the MHC allele. The peptides are useful to elicit an immune response against a desired antigen.

HLA BINDING PEPTIDES AND THEIR USES

The present application is a continuation in part of USSN 60/013,833, which is related to USSN 08/589,107, and USSN 08/451,913 and to USSN 08/347,610, which is a continuation in part of USSN 08/159,339, which is continuation in part of USSN 08/103,396 which is a continuation in part of USSN 08/027,746 which is a continuation in part of USSN 07/926,666. It is also related to USSN 08/186,266. All of the above applications are incorporated herein by reference.

BACKGROUND OF THE INVENTION

The present invention relates to compositions and methods for preventing, treating or diagnosing a number of pathological states such as viral diseases and cancers. In particular, it provides novel peptides capable of binding selected major histocompatibility complex (MHC) molecules and inducing an immune response.

MHC molecules are classified as either Class I or Class II molecules. Class II MHC molecules are expressed primarily on cells involved in initiating and sustaining immune responses, such as T lymphocytes, B lymphocytes, macrophages, etc. Class II MHC molecules are recognized by helper T lymphocytes and induce proliferation of helper T lymphocytes and amplification of the immune response to the particular immunogenic peptide that is displayed. Class I MHC molecules are expressed on almost all nucleated cells and are recognized by cytotoxic T lymphocytes (CTLs), which then destroy the antigen-bearing cells. CTLs are particularly important in tumor rejection and in fighting viral infections. The CTL recognizes the antigen in the form of a peptide fragment bound to the MHC class I molecules rather than the intact foreign antigen itself. The antigen must normally be endogenously synthesized by the cell, and a portion of the protein antigen is degraded into small peptide fragments in the cytoplasm. Some of these small peptides translocate into a pre-Golgi compartment and interact with class I heavy chains to facilitate proper folding and association with the

subunit β2 microglobulin. The peptide-MHC class 1 complex is then routed to the cell surface for expression and potential recognition by specific CTLs.

Investigations of the crystal structure of the human MHC class I molecule, HLA-A2.1, indicate that a peptide binding groove is created by the folding of the α1 and α2 domains of the class I heavy chain (Bjorkman et al., Nature 329:506 (1987). In these investigations, however, the identity of peptides bound to the groove was not determined.

Buus et al., Science 242:1065 (1988) first described a method for acid elution of bound peptides from MHC. Subsequently, Rammensee and his coworkers (Falk et al., Nature 351:290 (1991) have developed an approach to characterize naturally processed peptides bound to class I molecules. Other investigators have successfully achieved direct amino acid sequencing of the more abundant peptides in various HPLC fractions by conventional automated sequencing of peptides eluted from class I molecules of the B type (Jardetzky, et al., Nature 353:326 (1991) and of the A2.1 type by mass spectrometry (Hunt, et al., Science 225:1261 (1992). A review of the characterization of naturally processed peptides in MHC Class I has been presented by Rötzschke and Falk (Rötzschke and Falk, Immunol. Today 12:447 (1991).

Sette et al., Proc. Natl. Acad. Sci. USA 86:3296 (1989) showed that MHC allele specific motifs could be used to predict MHC binding capacity. Schaeffer et al., Proc. Natl. Acad. Sci. USA 86:4649 (1989) showed that MHC binding was related to immunogenicity. Several authors (De Bruijn et al., Eur. J. Immunol., 21:2963-2970 (1991); Pamer et al., 991 Nature 353:852-955 (1991)) have provided preliminary evidence that class I binding motifs can be applied to the identification of potential immunogenic peptides in animal models. Class I motifs specific for a number of human alleles of a given class I isotype have yet to be described. It is desirable that the combined frequencies of these different alleles should be high enough to cover a large fraction or perhaps the majority of the human outbred population.

Despite the developments in the art, the prior art has yet to provide a useful human peptide-based vaccine or therapeutic agent based on this work. The present invention provides these and other advantages.

SUMMARY OF THE INVENTION

The present invention immunogenic peptides having binding motifs for MHC Class I molecules. The immunogenic peptides are typically between about 8 and about 11 residues and comprise conserved residues involved in binding proteins encoded by the appropriate MHC allele. A number of allele specific motifs have been identified.

For instance, the motif for HLA-A3.2 comprises from the N-terminus to C-terminus a first conserved residue of L, M, I, V, S, A, T and F at position 2 and a second conserved residue of K, R or Y at the C-terminal end. Other first conserved residues are C, G or D and alternatively E. Other second conserved residues are H or F. The first and second conserved residues are preferably separated by 6 to 7 residues.

The motif for HLA-A1 comprises from the N-terminus to the C-terminus a first conserved residue of T, S or M, a second conserved residue of D or E, and a third conserved residue of Y. Other second conserved residues are A, S or T. The first and second conserved residues are adjacent and are preferably separated from the third conserved residue by 6 to 7 residues. A second motif consists of a first conserved residue of E or D and a second conserved residue of Y where the first and second conserved residues are separated by 5 to 6 residues.

The motif for HLA-A11 comprises from the N-terminus to the C-terminus a first conserved residue of T or V at position 2 and a C-terminal conserved residue of K. The first and second conserved residues are preferably separated by 6 or 7 residues.

The motif for HLA-A24.1 comprises from the N-terminus to the C-terminus a first conserved residue of Y, F or W at position 2 and a C terminal conserved residue of F, I, W, M or L. The first and second conserved residues are preferably separated by 6 to 7 residues.

Epitopes on a number of potential target proteins can be identified in this manner. The peptides can be prepared based on sequences of antigenic proteins from pathogens (e.g., viral pathogens, fungal pathogens, bacterial pathogens, protozoal pathogens, and the like) or from antigens associated with cancer. Examples of suitable antigens include prostate specific antigen (PSA), hepatitis B core and surface antigens (HBVc, HBVs) hepatitis C antigens, malignant melanoma antigen (MAGE-1) Epstein-Barr virus antigens, human immunodeficiency type-1 virus (HIV1) and papilloma virus

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antigens. The peptides or nucleic acids that encode them are useful in pharmaceutical compositions for both in vivo and ex vivo therapeutic and diagnostic applications.

Definitions

The term "peptide" is used interchangeably with "oligopeptide" in the present specification to designate a series of residues, typically L-amino acids, connected one to the other typically by peptide bonds between the alpha-amino and carbonyl groups of adjacent amino acids. The oligopeptides of the invention are less than about 15 residues in length and usually consist of between about 8 and about 11 residues, preferably 9 or 10 residues.

An "immunogenic peptide" is a peptide which comprises an allele-specific motif such that the peptide will bind the MHC allele and be capable of inducing a CTL response. Thus, immunogenic peptides are capable of binding to an appropriate class I MHC molecule and inducing a cytotoxic T cell response against the antigen from which the immunogenic peptide is derived.

The relationship between binding affinity for MHC class I molecules and immunogenicity of discrete peptide epitopes has been analyzed in two different experimental approaches (Sette, et al., J. Immunol., 153:5586-5592 (1994)). In the first approach, the immunogenicity of potential epitopes ranging in MHC binding affinity over a 10,000-fold range was analyzed in HLA-A*0201 transgenic mice. In the second approach, the antigenicity of approximately 100 different hepatitis B virus (HBV)-derived potential epitopes, all carrying A*0201 binding motifs, was assessed by using PBL of acute hepatitis patients. In both cases, it was found that an affinity threshold of approximately 500 nM (preferably 500 nM or less) determines the capacity of a peptide epitope to elicit a CTL response. These data correlate well with class I binding affinity measurements of either naturally processed peptides or previously described T cell epitopes. These data indicate the important role of determinant selection in the shaping of T cell responses.

A "conserved residue" is an amino acid which occurs in a significantly higher frequency than would be expected by random distribution at a particular position in a peptide motif. Typically a conserved residue is one at which the immunogenic peptide may provide a contact point with the MHC molecule. One to three, preferably two, conserved residues within a peptide of defined length defines a motif for an immunogenic

peptide. These residues are typically in close contact with the peptide binding groove, with their side chains buried in specific pockets of the groove itself. Typically, an immunogenic peptide will comprise up to three conserved residues, more usually two conserved residues.

As used herein, "negative binding residues" are amino acids which if present at certain positions will result in a peptide being a nonbinder or poor binder and in turn fail to induce a CTL response despite the presence of the appropriate conserved residues within the peptide.

The term "motif" refers to the pattern of residues in a peptide of defined length, usually about 8 to about 11 amino acids, which is recognized by a particular MHC allele. The peptide motifs are typically different for each human MHC allele and differ in the pattern of the highly conserved residues.

The binding motif for an allele can be defined with increasing degrees of precision. In one case, all of the conserved residues are present in the correct positions in a peptide and there are no negative binding residues present.

The phrases "isolated" or "biologically pure" refer to material which is substantially or essentially free from components which normally accompany it as found in its native state. Thus, the peptides of this invention do not contain materials normally associated with their in situ environment, e.g., MHC I molecules on antigen presenting cells. Even where a protein has been isolated to a homogenous or dominant band, there are trace contaminants in the range of 5-10% of native protein which co-purify with the desired protein. Isolated peptides of this invention do not contain such endogenous co-purified protein.

The term "residue" refers to an amino acid or amino acid mimetic incorporated in a oligopeptide by an amide bond or amide bond mimetic.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention relates to the determination of allele-specific peptide motifs for human Class I MHC (sometimes referred to as HLA) allele subtypes. These motifs are then used to define T cell epitopes from any desired antigen, particularly those associated with human viral diseases, cancers or autoimmune diseases, for which the amino acid sequence of the potential antigen or autoantigen targets is known.

Epitopes on a number of potential target proteins can be identified in this manner. Examples of suitable antigens include prostate specific antigen (PSA), hepatitis B core and surface antigens (HBVc, HBVs) hepatitis C antigens, Epstein-Barr virus antigens, melanoma antigens (e.g., MAGE-1), human immunodeficiency virus (HIV) antigens and human papilloma virus (HPV) antigens.

Autoimmune associated disorders for which the peptides of the invention may be employed to relieve the symptoms of, treat or prevent the occurrence or reoccurrence of include, for example, multiple sclerosis (MS), rheumatoid arthritis (RA), Sjogren syndrome, scleroderma, polymyositis, dermatomyositis, systemic lupus erythematosus, juvenile rheumatoid arthritis, ankylosing spondylitis, myasthenia gravis (MG), bullous pemphigoid (antibodies to basement membrane at dermal-epidermal junction), pemphigus (antibodies to mucopolysaccharide protein complex or intracellular cement substance), glomerulonephritis (antibodies to glomerular basement membrane), Goodpasture's syndrome, autoimmune hemolytic anemia (antibodies to erythrocytes), Hashimoto's disease (antibodies to thyroid), pernicious anemia (antibodies to intrinsic factor), idiopathic thrombocytopenic purpura (antibodies to platelets), Grave's disease, and Addison's disease (antibodies to thyroglobulin), and the like.

The autoantigens associated with a number of these diseases have been identified. For example, in experimentally induced autoimmune diseases, antigens involved in pathogenesis have been characterized: in arthritis in rat and mouse, native type-II collagen is identified in collagen-induced arthritis, and mycobacterial heat shock protein in adjuvant arthritis; thyroglobulin has been identified in experimental allergic thyroiditis (EAT) in mouse; acetyl choline receptor (AChR) in experimental allergic myasthenia gravis (EAMG); and myelin basic protein (MBP) and proteolipid protein (PLP) in experimental allergic encephalomyelitis (EAE) in mouse and rat. In addition, target antigens have been identified in humans: type-II collagen in human rheumatoid arthritis; and acetyl choline receptor in myasthenia gravis.

Without wishing to be bound by theory, it is believed that the presentation of antigen by HLA Class I mediates suppression of autoreactive T cells by CD8^{+} suppressor T cells (see, e.g., Jiang et al. Science 256:1213 (1992)). Such suppressor T cells release cytokines such as transforming growth factor-β (TGF-β), which specifically

inhibit the autoreactive T cells. Miller *et al. Proc. Natl. Acad. Sci. USA* 89:421-425
(1992).

Peptides comprising the epitopes from these antigens are synthesized and
then tested for their ability to bind to the appropriate MHC molecules in assays using, for
example, purified class I molecules and radioiodonated peptides and/or cells expressing
empty class I molecules by, for instance, immunofluorescent staining and flow
microfluorimetry, peptide-dependent class I assembly assays, and inhibition of CTL
recognition by peptide competition. Those peptides that bind to the class I molecule are
further evaluated for their ability to serve as targets for CTLs derived from infected or
immunized individuals, as well as for their capacity to induce primary in vitro or in vivo
CTL responses that can give rise to CTL populations capable of reacting with virally
infected target cells or tumor cells as potential therapeutic agents.

The MHC class I antigens are encoded by the HLA-A, B, and C loci.
HLA-A and B antigens are expressed at the cell surface at approximately equal densities,
whereas the expression of HLA-C is significantly lower (perhaps as much as 10-fold
lower). Each of these loci have a number of alleles. The peptide binding motifs of the
invention are relatively specific for each allelic subtype.

For peptide-based vaccines, the peptides of the present invention preferably
comprise a motif recognized by an MHC I molecule having a wide distribution in the
human population. Since the MHC alleles occur at different frequencies within different
ethnic groups and races, the choice of target MHC allele may depend upon the target
population. Table 1 shows the frequency of various alleles at the HLA-A locus products
among different races. For instance, the majority of the Caucasoid population can be
covered by peptides which bind to four HLA-A allele subtypes, specifically HLA-A2.1,
A1, A3.2, and A24.1. Similarly, the majority of the Asian population is encompassed
with the addition of peptides binding to a fifth allele HLA-A11.2.

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TABLE 1

A Allele/Subtype	N(69)[*]	A(54)	C(502)
A1	10.1(7)	1.8(1)	27.4(138)
A2.1	11.5(8)	37.0(20)	39.8(199)
A2.2	10.1(7)	0	3.3(17)
A2.3	1.4(1)	5.5(3)	0.8(4)
A2.4	-	-	-
A2.5	-	-	-
A3.1	1.4(1)	0	0.2(0)
A3.2	5.7(4)	5.5(3)	21.5(108)
A11.1	0	5.5(3)	0
A11.2	5.7(4)	31.4(17)	8.7(44)
A11.3	0	3.7(2)	0
A23	4.3(3)	-	3.9(20)
A24	2.9(2)	27.7(15)	15.3(77)
A24.2	-	-	-
A24.3	-	-	-
A25	1.4(1)	-	6.9(35)
A26.1	4.3(3)	9.2(5)	5.9(30)
A26.2	7.2(5)	-	1.0(5)
A26V	-	3.7(2)	-
A28.1	10.1(7)	-	1.6(8)
A28.2	1.4(1)	-	7.5(38)
A29.1	1.4(1)	-	1.4(7)
A29.2	10.1(7)	1.8(1)	5.3(27)
A30.1	8.6(6)	-	4.9(25)
A30.2	1.4(1)	-	0.2(1)
A30.3	7.2(5)	-	3.9(20)
A31	4.3(3)	7.4(4)	6.9(35)
A32	2.8(2)	-	7.1(36)
Aw33.1	8.6(6)	-	2.5(13)
Aw33.2	2.8(2)	16.6(9)	1.2(6)
Aw34.1	1.4(1)	-	-
Aw34.2	14.5(10)	-	0.8(4)
Aw36	5.9(4)	-	-

Tab. compiled from B. DuPont, Immunobiology of HLA, Vol. I, Histocompatibility
Testing 1987, Springer-Verlag, New York 1989.

[*] N - negroid; A = Asian; C = caucasoid. Numbers in
parenthesis represent the number of individuals included in the analysis.

The nomenclature used to describe peptide compounds follows the
conventional practice wherein the amino group is presented to the left (the N-terminus) and

the carboxyl group to the right (the C-terminus) of each amino acid residue. In the formulae representing selected specific embodiments of the present invention, the amino- and carboxyl-terminal groups, although not specifically shown, are in the form they would assume at physiologic pH values, unless otherwise specified. In the amino acid structure formulae, each residue is generally represented by standard three letter or single letter designations. The L-form of an amino acid residue is represented by a capital single letter or a capital first letter of a three-letter symbol, and the D-form for those amino acids is represented by a lower case single letter or a lower case three letter symbol. Glycine has no asymmetric carbon atom and is simply referred to as "Gly" or G.

The procedures used to identify peptides of the present invention generally follow the methods disclosed in Falk et al., Nature 351:290 (1991), which is incorporated herein by reference. Briefly, the methods involve large-scale isolation of MHC class I molecules, typically by immunoprecipitation or affinity chromatography, from the appropriate cell or cell line. Examples of other methods for isolation of the desired MHC molecule equally well known to the artisan include ion exchange chromatography, lectin chromatography, size exclusion, high performance ligand chromatography, and a combination of all of the above techniques.

A large number of cells with defined MHC molecules, particularly MHC Class I molecules, are known and readily available. For example, human EBV-transformed B cell lines have been shown to be excellent sources for the preparative isolation of class I and class II MHC molecules. Well-characterized cell lines are available from private and commercial sources, such as American Type Culture Collection ("Catalogue of Cell Lines and Hybridomas," 6th edition (1988) Rockville, Maryland, U.S.A.); National Institute of General Medical Sciences 1990/1991 Catalog of Cell Lines (NIGMS) Human Genetic Mutant Cell Repository, Camden, NJ; and ASHI Repository, Bingham and Women's Hospital, 75 Francis Street, Boston, MA 02115. Table 2 lists some B cell lines suitable for use as sources for HLA-A alleles. All of these cell lines can be grown in large batches and are therefore useful for large scale production of MHC molecules. One of skill will recognize that these are merely exemplary cell lines and that many other cell sources can be employed. Similar EBV B cell lines homozygous for HLA-B and HLA-C could serve as sources for HLA-B and HLA-C alleles, respectively.

TABLE 2

HUMAN CELL LINES (HLA-A SOURCES)

HLA-A allele	B cell line
A1	MAT COX (9022) STEINLIN (9087)
A2.1	JY
A3.2	EHM (9080) HO301 (9055)GM3107
A24.1	KT3(9107),TISI (9042)
A11	BVR (GM6828A) WT100 (GM8602)WT52 (GM8603)

In the typical case, immunoprecipitation is used to isolate the desired allele. A number of protocols can be used, depending upon the specificity of the antibodies used. For example, allele-specific mAb reagents can be used for the affinity purification of the HLA-A, HLA-B, and HLA-C molecules. Several mAb reagents for the isolation of HLA-A molecules are available (Table 3). Thus, for each of the targeted HLA-A alleles, reagents are available that may be used for the direct isolation of the HLA-A molecules.

Affinity columns prepared with these mAbs using standard techniques are successfully

used to purify the respective HLA-A allele products.

In addition to allele-specific mAbs, broadly

reactive anti-HLA-A, B, C mAbs, such as W6/32 and B9.12.1, and one anti-HLA-B, C

5   mAb, B1.23.2, could be used in alternative affinity purification protocols as described in

the example section below.

TABLE 3

ANTIBODY REAGENTS

anti-HLA	Name	
HLA-A1	12/18	
HLA-A3	GAPA3	(ATCC, HB122)
HLA-11,24.1	A11.1M	(ATCC, HB164)
HLA-A,B,C	W6/32	(ATCC, HB95)
monomorphic	B9.12.1	(INSERM-CNRS)
HLA-B,C	B.1.23.2	(INSERM-CNRS)
monomorphic		

The peptides bound to the peptide binding groove of the isolated MHC

20   molecules are eluted typically using acid treatment. Peptides can also be dissociated from

class I molecules by a variety of standard denaturing means, such as heat, pH, detergents,

salts, chaotropic agents, or a combination thereof.

Peptide fractions are further separated from the MHC molecules by

reversed-phase high performance liquid chromatography (HPLC) and sequenced. Peptides

25   can be separated by a variety of other standard means well known to the artisan, including

filtration, ultrafiltration, electrophoresis, size chromatography, precipitation with specific

antibodies, ion exchange chromatography, isoelectrofocusing, and the like.

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Sequencing of the isolated peptides can be performed according to standard techniques such as Edman degradation (Hunkapiller, M.W., et al., Methods Enzymol. 91, 399 [1983]). Other methods suitable for sequencing include mass spectrometry sequencing of individual peptides as previously described (Hunt, et al., Science 225:1261 (1992), which is incorporated herein by reference). Amino acid sequencing of bulk heterogenous peptides (e.g., pooled HPLC fractions) from different class I molecules typically reveals a characteristic sequence motif for each class I allele.

Definition of motifs specific for different class I alleles allows the identification of potential peptide epitopes from an antigenic protein whose amino acid sequence is known. Typically, identification of potential peptide epitopes is initially carried out using a computer to scan the amino acid sequence of a desired antigen for the presence of motifs. The epitopic sequences are then synthesized. The capacity to bind MHC Class molecules is measured in a variety of different ways. One means is a Class I molecular binding assay as described, for instance, in the related applications, noted above. Other alternatives described in the literature include inhibition of antigen presentation (Sette, et al., J. Immunol. 141:3893 (1991), in vitro assembly assays (Townsend, et al., Cell 62:285 (1990), and FACS based assays using mutated cells, such as RMA.S (Melief, et al., Eur. J. Immunol. 21:2963 [1991]).

Next, peptides that test positive in the MHC class I binding assay are assayed for the ability of the peptides to induce specific CTL responses in vitro. For instance, antigen-presenting cells that have been incubated with a peptide can be assayed for the ability to induce CTL responses in responder cell populations. Antigen-presenting cells can be normal cells such as peripheral blood mononuclear cells or dendritic cells (Inaba, et al., J. Exp. Med. 166:182 (1987); Boog, Eur. J. Immunol. 18:219 [1988]).

Alternatively, mutant mammalian cell lines that are deficient in their ability to load class I molecules with internally processed peptides, such as the mouse cell lines RMA-S (Kärre, et al., Nature, 319:675 (1986); Ljunggren, et al., Eur. J. Immunol. 21:2963-2970 (1991)), and the human somatic T cell hybridoma, T-2 (Cerundolo, et al., Nature 345:449-452 (1990)) and which have been transfected with the appropriate human class I genes are conveniently used, when peptide is added to them, to test for the capacity of the peptide to induce in vitro primary CTL responses. Other eukaryotic cell lines which could be used include various insect cell lines such as mosquito larvae (ATCC cell lines

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CCL 125, 126, 1660, 1591, 6585, 6586), silkworm (ATTC CRL 8851), armyworm (ATCC CRL 1711), moth (ATCC CCL 80) and Drosophila cell lines such as a Schneider cell line (see Schneider J. Embryol. Exp. Morphol. 27:353-365 [1927]). That have been transfected with the appropriate human class I MHC allele encoding genes and the human B2 microglobulin genes.

Peripheral blood lymphocytes are conveniently isolated following simple venipuncture or leukapheresis of normal donors or patients and used as the responder cell sources of CTL precursors. In one embodiment, the appropriate antigen-presenting cells are incubated with 10-100 μM of peptide in serum-free media for 4 hours under appropriate culture conditions. The peptide-loaded antigen-presenting cells are then incubated with the responder cell populations in vitro for 7 to 10 days under optimized culture conditions. Positive CTL activation can be determined by assaying the cultures for the presence of CTLs that kill radiolabeled target cells, both specific peptide-pulsed targets as well as target cells expressing endogenously processed form of the relevant virus or tumor antigen from which the peptide sequence was derived.

Specificity and MHC restriction of the CTL is determined by testing against different peptide target cells expressing appropriate or inappropriate human MHC class I. The peptides that test positive in the MHC binding assays and give rise to specific CTL responses are referred to herein as immunogenic peptides.

The immunogenic peptides can be prepared synthetically, or by recombinant DNA technology or isolated from natural sources such as whole viruses or tumors. Although the peptide will preferably be substantially free of other naturally occurring host cell proteins and fragments thereof, in some embodiments the peptides can be synthetically conjugated to native fragments or particles. The polypeptides or peptides can be a variety of lengths, either in their neutral (uncharged) forms or in forms which are salts, and either free of modifications such as glycosylation, side chain oxidation, or phosphorylation or containing these modifications, subject to the condition that the modification not destroy the biological activity of the polypeptides as herein described.

Desirably, the peptide will be as small as possible while still maintaining substantially all of the biological activity of the large peptide. When possible, it may be desirable to optimize peptides of the invention to a length of 9 or 10 amino acid residues,

commensurate in size with endogenously processed viral peptides or tumor cell peptides that are bound to MHC class I molecules on the cell surface.

Peptides having the desired activity may be modified as necessary to provide
5   certain desired attributes, e.g., improved pharmacological characteristics, while increasing or at least retaining substantially all of the biological activity of the unmodified peptide to bind the desired MHC molecule and activate the appropriate T cell. For instance, the peptides may be subject to various changes, such as substitutions, either conservative or nonconservative, where such changes might provide for certain advantages in their use,
10 such as improved MHC binding. By conservative substitutions is meant replacing an amino acid residue with another which is biologically and/or chemically similar, e.g., one hydrophobic residue for another, or one polar residue for another. The substitutions include combinations such as Gly, Ala; Val, Ile, Leu, Met; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr. The effect of single amino acid substitutions may also be probed using D-amino acids. Such modifications may be made using well known peptide
15 synthesis procedures, as described in e.g., Merrifield, Science 232:341-347 (1986), Barany and Merrifield, The Peptides, Gross and Meienhofer, eds. (N.Y., Academic Press), pp. 1-284 (1979); and Stewart and Young, Solid Phase Peptide Synthesis, (Rockford, Ill., Pierce), 2d Ed. (1984), incorporated by reference herein.

The peptides can also be modified by extending or decreasing the
20 compound's amino acid sequence, e.g., by the addition or deletion of amino acids. The peptides or analogs of the invention can also be modified by altering the order or composition of certain residues, it being readily appreciated that certain amino acid residues essential for biological activity, e.g., those at critical contact sites or conserved residues, may generally not be altered without an adverse effect on biological activity.
25 The noncritical amino acids need not be limited to those naturally occurring in proteins, such as L-α-amino acids, or their D-isomers, but may include non-natural amino acids as well, such as β-γ-δ-amino acids, as well as many derivatives of L-α-amino acids.

Typically, a series of peptides with single amino acid substitutions is employed to determine the effect of electrostatic charge, hydrophobicity, etc. on binding.
30 For instance, a series of positively charged (e.g., Lys or Arg) or negatively charged (e.g., Glu) amino acid substitutions are made along the length of the peptide revealing different patterns of sensitivity towards various MHC molecules and T cell receptors. In addition,

multiple substitutions using small, relatively neutral moieties such as Ala, Gly, Pro, or similar residues may be employed. The substitutions may be homo-oligomers or hetero-oligomers. The number and types of residues which are substituted or added depend on the spacing necessary between essential contact points and certain functional attributes which are sought (e.g., hydrophobicity versus hydrophilicity). Increased binding affinity for an MHC molecule or T cell receptor may also be achieved by such substitutions, compared to the affinity of the parent peptide. In any event, such substitutions should employ amino acid residues or other molecular fragments chosen to avoid, for example, steric and charge interference which might disrupt binding.

Amino acid substitutions are typically of single residues. Substitutions, deletions, insertions or any combination thereof may be combined to arrive at a final peptide. Substitutional variants are those in which at least one residue of a peptide has been removed and a different residue inserted in its place. Such substitutions generally are made in accordance with the following Table 4 when it is desired to finely modulate the characteristics of the peptide.

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TABLE 4

<u>Original Residue</u>	<u>Exemplary Substitution</u>
Ala	ser
Arg	lys
Asn	gln; his
Asp	glu
Cys	ser
Gln	asn
Glu	asp
Gly	pro
His	asn; gln
Ile	leu; val
Leu	ile; val
Lys	arg
Met	leu; ile
Phe	met; leu; tyr
Ser	thr
Thr	ser
Trp	tyr
Tyr	trp; phe
Val	ile; leu

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Substantial changes in function (e.g., affinity for MHC molecules or T cell
receptors) are made by selecting substitutions that are less conservative than those in Table
4, i.e., selecting residues that differ more significantly in their effect on maintaining (a)
the structure of the peptide backbone in the area of the substitution, for example as a sheet

5   or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site
or (c) the bulk of the side chain. The substitutions which in general are expected to
produce the greatest changes in peptide properties will be those in which (a) hydrophilic
residue, e.g. seryl, is substituted for (or by) a hydrophobic residue, e.g. leucyl, isoleucyl,
phenylalanyl, valyl or alanyl; (b) a residue having an electropositive side chain, e.g.,

10   lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g.
glutamyl or aspartyl; or (c) a residue having a bulky side chain, e.g. phenylalanine, is
substituted for (or by) one not having a side chain, e.g., glycine.

The peptides may also comprise isosteres of two or more residues in the
immunogenic peptide. An isostere as defined here is a sequence of two or more residues

15   that can be substituted for a second sequence because the steric conformation of the first
sequence fits a binding site specific for the second sequence. The term specifically
includes peptide backbone modifications well known to those skilled in the art. Such
modifications include modifications of the amide nitrogen, the α-carbon, amide carbonyl,
complete replacement of the amide bond, extensions, deletions or backbone crosslinks.

20   See, generally, Spatola, Chemistry and Biochemistry of Amino Acids, peptides and
Proteins, Vol. VII (Weinstein ed., 1983).

Modifications of peptides with various amino acid mimetics or unnatural
amino acids are particularly useful in increasing the stability of the peptide in vivo.
Stability can be assayed in a number of ways. For instance, peptidases and various

25   biological media, such as human plasma and serum, have been used to test stability. See,
e.g., Verhoef et al., Eur. J. Drug Metab Pharmacokin. 11:291-302 (1986). Half life of
the peptides of the present invention is conveniently determined using a 25% human serum
(v/v) assay. The protocol is generally as follows. Pooled human serum (Type AB,
non-heat inactivated) is delipidated by centrifugation before use. The serum is then diluted

30   to 25% with RPMI tissue culture media and used to test peptide stability. At
predetermined time intervals a small amount of reaction solution is removed and added to
either 6% aqueous trichloracetic acid or ethanol. The cloudy reaction sample is cooled

(4°C) for 15 minutes and then spun to pellet the precipitated serum proteins. The presence of the peptides is then determined by reversed-phase HPLC using stability-specific chromatography conditions.

The peptides of the present invention or analogs thereof which have CTL
5   stimulating activity may be modified to provide desired attributes other than improved serum half life. For instance, the ability of the peptides to induce CTL activity can be enhanced by linkage to a sequence which contains at least one epitope that is capable of inducing a T helper cell response. Particularly preferred immunogenic peptides/T helper conjugates are linked by a spacer molecule. The spacer is typically comprised of relatively
10 small, neutral molecules, such as amino acids or amino acid mimetics, which are substantially uncharged under physiological conditions. The spacers are typically selected from, e.g., Ala, Gly, or other neutral spacers of nonpolar amino acids or neutral polar amino acids. It will be understood that the optionally present spacer need not be comprised of the same residues and thus may be a hetero- or homo-oligomer. When
15 present, the spacer will usually be at least one or two residues, more usually three to six residues. Alternatively, the CTL peptide may be linked to the T helper peptide without a spacer.

The immunogenic peptide may be linked to the T helper peptide either directly or via a spacer either at the amino or carboxy terminus of the CTL peptide. The amino
20 terminus of either the immunogenic peptide or the T helper peptide may be acylated.

In some embodiments it may be desirable to include in the pharmaceutical compositions of the invention at least one component which assists in priming CTL. Lipids have been identified as agents capable of assisting the priming CTL in vivo against viral antigens. For example, palmitic acid residues can be attached to the alpha and
25 epsilon amino groups of a Lys residue and then linked, e.g., via one or more linking residues such as Gly, Gly-Gly-, Ser, Ser-Ser, or the like, to an immunogenic peptide. The lipidated peptide can then be injected directly in a micellar form, incorporated into a liposome or emulsified in an adjuvant, e.g., incomplete Freund's adjuvant. In a preferred embodiment a particularly effective immunogen comprises palmitic acid attached to alpha
30 and epsilon amino groups of Lys, which is attached via linkage, e.g., Ser-Ser, to the amino terminus of the immunogenic peptide.

As another example of lipid priming of CTL responses, E. coli lipoproteins, such as tripalmitoyl-S-glycerylcysteinlyseryl-serine (P₃CSS) can be used to prime virus specific CTL when covalently attached to an appropriate peptide. See, Deres et al., Nature 342:561-564 (1989), incorporated herein by reference. Peptides of the invention can be coupled to P₃CSS, for example, and the lipopeptide administered to an individual to specifically prime a CTL response to the target antigen. Further, as the induction of neutralizing antibodies can also be primed with P₃CSS conjugated to a peptide which displays an appropriate epitope, the two compositions can be combined to more effectively elicit both humoral and cell-mediated responses to infection.

In addition, additional amino acids can be added to the termini of a peptide to provide for ease of linking peptides one to another, for coupling to a carrier support, or larger peptide, for modifying the physical or chemical properties of the peptide or oligopeptide, or the like. Amino acids such as tyrosine, cysteine, lysine, glutamic or aspartic acid, or the like, can be introduced at the C- or N-terminus of the peptide or oligopeptide. Modification at the C terminus in some cases may alter binding characteristics of the peptide. In addition, the peptide or oligopeptide sequences can differ from the natural sequence by being modified by terminal-NH₂ acylation, e.g., by alkanoyl (C_1-C_{20}) or thioglycolyl acetylation, terminal-carboxyl amidation, e.g., ammonia, methylamine, etc. In some instances these modifications may provide sites for linking to a support or other molecule.

The peptides of the invention can be prepared in a wide variety of ways. Because of their relatively short size, the peptides can be synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. See, for example, Stewart and Young, Solid Phase Peptide Synthesis, 2d. ed., Pierce Chemical Co. (1984) supra.

Alternatively, recombinant DNA technology may be employed wherein a nucleotide sequence which encodes an immunogenic peptide of interest is inserted into an expression vector, transformed or transfected into an appropriate host cell and cultivated under conditions suitable for expression. These procedures are generally known in the art, as described generally in Sambrook et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, New York (1982), which is incorporated herein

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by reference. Thus, fusion proteins which comprise one or more peptide sequences of the invention can be used to present the appropriate T cell epitope.

As the coding sequence for peptides of the length contemplated herein can be synthesized by chemical techniques, for example, the phosphotriester method of Matteucci

5   et al., J. Am. Chem. Soc. 103:3185 (1981), modification can be made simply by substituting the appropriate base(s) for those encoding the native peptide sequence. The coding sequence can then be provided with appropriate linkers and ligated into expression vectors commonly available in the art, and the vectors used to transform suitable hosts to produce the desired fusion protein. A number of such vectors and suitable host systems

10   are now available. For expression of the fusion proteins, the coding sequence will be provided with operably linked start and stop codons, promoter and terminator regions and usually a replication system to provide an expression vector for expression in the desired cellular host. For example, promoter sequences compatible with bacterial hosts are provided in plasmids containing convenient restriction sites for insertion of the desired

15   coding sequence. The resulting expression vectors are transformed into suitable bacterial hosts. Of course, yeast or mammalian cell hosts may also be used, employing suitable vectors and control sequences.

The peptides of the present invention and pharmaceutical and vaccine compositions thereof are useful for administration to mammals, particularly humans, to

20   treat and/or prevent viral infection and cancer. Examples of diseases which can be treated using the immunogenic peptides of the invention include prostate cancer, hepatitis B, hepatitis C, AIDS, renal carcinoma, cervical carcinoma, lymphoma, CMV and condlyloma acuminatum.

For pharmaceutical compositions, the immunogenic peptides of the invention

25   are administered to an individual already suffering from cancer or infected with the virus of interest. Those in the incubation phase or the acute phase of infection can be treated with the immunogenic peptides separately or in conjunction with other treatments, as appropriate. In therapeutic applications, compositions are administered to a patient in an amount sufficient to elicit an effective CTL response to the virus or tumor antigen and to

30   cure or at least partially arrest symptoms and/or complications. An amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on, e.g., the peptide composition, the manner of administration, the stage

and severity of the disease being treated, the weight and general state of health of the patient, and the judgment of the prescribing physician, but generally range for the initial immunization (that is for therapeutic or prophylactic administration) from about 1.0 μg to about 5000 μg of peptide for a 70 kg patient, followed by boosting dosages of from about

5   1.0 μg to about 1000 μg of peptide pursuant to a boosting regimen over weeks to months depending upon the patient's response and condition by measuring specific CTL activity in the patient's blood. It must be kept in mind that the peptides and compositions of the present invention may generally be employed in serious disease states, that is, life-threatening or potentially life threatening situations. In such cases, in view of the

10   minimization of extraneous substances and the relative nontoxic nature of the peptides, it is possible and may be felt desirable by the treating physician to administer substantial excesses of these peptide compositions.

For therapeutic use, administration should begin at the first sign of viral infection or the detection or surgical removal of tumors or shortly after diagnosis in the

15   case of acute infection. This is followed by boosting doses until at least symptoms are substantially abated and for a period thereafter. In chronic infection, loading doses followed by boosting doses may be required.

Treatment of an infected individual with the compositions of the invention may hasten resolution of the infection in acutely infected individuals. For those individuals

20   susceptible (or predisposed) to developing chronic infection the compositions are particularly useful in methods for preventing the evolution from acute to chronic infection. Where the susceptible individuals are identified prior to or during infection, for instance, as described herein, the composition can be targeted to them, minimizing need for administration to a larger population.

25   The peptide compositions can also be used for the treatment of chronic infection and to stimulate the immune system to eliminate virus-infected cells in carriers. It is important to provide an amount of immuno-potentiating peptide in a formulation and mode of administration sufficient to effectively stimulate a cytotoxic T cell response. Thus, for treatment of chronic infection, a representative dose is in the range of about 1.0

30   μg to about 5000 μg, preferably about 5 μg to 1000 μg for a 70 kg patient per dose. Immunizing doses followed by boosting doses at established intervals, e.g., from one to four weeks, may be required, possibly for a prolonged period of time to effectively

immunize an individual. In the case of chronic infection, administration should continue until at least clinical symptoms or laboratory tests indicate that the viral infection has been eliminated or substantially abated and for a period thereafter.

The pharmaceutical compositions for therapeutic treatment are intended for
5   parenteral, topical, oral or local administration. Preferably, the pharmaceutical compositions are administered parenterally, e.g., intravenously, subcutaneously, intradermally, or intramuscularly. Thus, the invention provides compositions for parenteral administration which comprise a solution of the immunogenic peptides dissolved or suspended in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous
10   carriers may be used, e.g., water, buffered water, 0.9% saline, 0.3% glycine, hyaluronic acid and the like. These compositions may be sterilized by conventional, well known sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration. The compositions may contain pharmaceutically
15   acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, etc.

The concentration of CTL stimulatory peptides of the invention in the
20   pharmaceutical formulations can vary widely, i.e., from less than about 0.1%, usually at or at least about 2% to as much as 20% to 50% or more by weight, and will be selected primarily by fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

The peptides of the invention may also be administered via liposomes, which
25   target the peptides to a particular cells tissue, such as lymphoid tissue. Liposomes are also useful in increasing the half-life of the peptides. Liposomes include emulsions, foams, micelles, insoluble monolayers, liquid crystals, phospholipid dispersions, lamellar layers and the like. In these preparations the peptide to be delivered is incorporated as part of a liposome, alone or in conjunction with a molecule which binds to, e.g., a receptor
30   prevalent among lymphoid cells, such as monoclonal antibodies which bind to the CD45 antigen, or with other therapeutic or immunogenic compositions. Thus, liposomes filled with a desired peptide of the invention can be directed to the site of lymphoid cells, where

the liposomes then deliver the selected therapeutic/immunogenic peptide compositions. Liposomes for use in the invention are formed from standard vesicle-forming lipids, which generally include neutral and negatively charged phospholipids and a sterol, such as cholesterol. The selection of lipids is generally guided by consideration of, e.g., liposome

5 size, acid lability and stability of the liposomes in the blood stream. A variety of methods are available for preparing liposomes, as described in, e.g., Szoka et al., Ann. Rev. Biophys. Bioeng. 9:467 (1980), U.S. Patent Nos. 4,235,871, 4,501,728, 4,837,028, and 5,019,369, incorporated herein by reference.

For targeting to the immune cells, a ligand to be incorporated into the

10 liposome can include, e.g., antibodies or fragments thereof specific for cell surface determinants of the desired immune system cells. A liposome suspension containing a peptide may be administered intravenously, locally, topically, etc. in a dose which varies according to, inter alia, the manner of administration, the peptide being delivered, and the stage of the disease being treated.

15 For solid compositions, conventional nontoxic solid carriers may be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as

20 those carriers previously listed, and generally 10-95% of active ingredient, that is, one or more peptides of the invention, and more preferably at a concentration of 25%-75%.

For aerosol administration, the immunogenic peptides are preferably supplied in finely divided form along with a surfactant and propellant. Typical percentages of peptides are 0.01%-20% by weight, preferably 1%-10%. The surfactant must, of course,

25 be nontoxic, and preferably soluble in the propellant. Representative of such agents are the esters or partial esters of fatty acids containing from 6 to 22 carbon atoms, such as caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric and oleic acids with an aliphatic polyhydric alcohol or its cyclic anhydride. Mixed esters, such as mixed or natural glycerides may be employed. The surfactant may constitute 0.1%-20% by weight

30 of the composition, preferably 0.25-5%. The balance of the composition is ordinarily propellant. A carrier can also be included, as desired, as with, e.g., lecithin for intranasal delivery.

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In another aspect the present invention is directed to vaccines which contain as an active ingredient an immunogenically effective amount of an immunogenic peptide as described herein. The peptide(s) may be introduced into a host, including humans, linked to its own carrier or as a homopolymer or heteropolymer of active peptide units. Such a
5   polymer has the advantage of increased immunological reaction and, where different peptides are used to make up the polymer, the additional ability to induce antibodies and/or CTLs that react with different antigenic determinants of the virus or tumor cells. Useful carriers are well known in the art, and include, e.g., thyroglobulin, albumins such as bovine serum albumin, tetanus toxoid, polyamino acids such as poly(lysine:glutamic
10   acid), hepatitis B virus core protein, hepatitis B virus recombinant vaccine and the like. The vaccines can also contain a physiologically tolerable (acceptable) diluent such as water, phosphate buffered saline, or saline, and further typically include an adjuvant. Adjuvants such as incomplete Freund's adjuvant, aluminum phosphate, aluminum hydroxide, or alum are materials well known in the art. And, as mentioned above, CTL
15   responses can be primed by conjugating peptides of the invention to lipids, such as P_3CSS. Upon immunization with a peptide composition as described herein, via injection, aerosol, oral, transdermal or other route, the immune system of the host responds to the vaccine by producing large amounts of CTLs specific for the desired antigen, and the host becomes at least partially immune to later infection, or resistant to developing chronic infection.
20   Vaccine compositions containing the peptides of the invention are administered to a patient susceptible to or otherwise at risk of viral infection or cancer to elicit an immune response against the antigen and thus enhance the patient's own immune response capabilities. Such an amount is defined to be an "immunogenically effective dose." In this use, the precise amounts again depend on the patient's state of health and weight, the
25   mode of administration, the nature of the formulation, etc., but generally range from about 1.0 μg to about 5000 μg per 70 kilogram patient, more commonly from about 10 μg to about 500 μg mg per 70 kg of body weight.

In some instances it may be desirable to combine the peptide vaccines of the invention with vaccines which induce neutralizing antibody responses to the virus of
30   interest, particularly to viral envelope antigens.

For therapeutic or immunization purposes, the

25

peptides of the invention can also be expressed by attenuated viral hosts, such as vaccinia or fowlpox. This approach involves the use of vaccinia virus as a vector to express nucleotide sequences that encode the peptides of the invention. Upon introduction into an acutely or chronically infected host or into a noninfected host, the recombinant vaccinia

5   virus expresses the immunogenic peptide, and thereby elicits a host CTL response. Vaccinia vectors and methods useful in immunization protocols are described in, e.g.,U.S. Patent No. 4,722,848, incorporated herein by reference. Another vector is BCG (Bacille Calmette Guerin). BCG vectors are described in Stover et al. (Nature 351:456-460 (1991)) which is incorporated herein by reference. A wide variety of other vectors useful

10   for therapeutic administration or immunization of the peptides of the invention, e.g., Salmonella typhi vectors and the like, will be apparent to those skilled in the art from the description herein.

A wide variety of other vectors useful
for therapeutic administration or immunization of the peptides of the invention, e.g.,

Nucleic acids encoding one or more of the peptides of the invention can also be admisitered to the patient. This approach is described, for instance, in Wolff et. al.,

15   Science 247: 1465-1468 (1990) as well as U.S. Patent Nos. 5,580,859 and 5,589,466.

A preferred means of administering nucleic acids encoding the peptides of the invention uses minigene constructs encoding multiple epitopes of the invention. To create a DNA sequence encoding the selected CTL epitopes (minigene) for expression in human cells, the amino acid sequences of the epitopes are reverse translated. A human codon

20   usage table is used to guide the codon choice for each amino acid. These epitope-encoding DNA sequences are directly adjoined, creating a continuous polypeptide sequence. To optimize expression and/or immunogenicity, additional elements can be incorporated into the minigene design. Examples of amino acid sequence that could be reverse translated and included in the minigene sequence include: helper T lymphocyte epitopes, a leader

25   (signal) sequence, and an endoplasmic reticulum retention signal. In addition, MHC presentation of CTL epitopes may be improved by including synthetic (e.g. poly-alanine) or naturally-occurring flanking sequences adjacent to the CTL epitopes.

The minigene sequence is converted to DNA by assembling oligonucleotides that encode the plus and minus strands of the minigene. Overlapping oligonucleotides (30-

30   100 bases long) are synthesized, phosphorylated, purified and annealed under appropriate conditions using well known techniques. he ends of the oligonucleotides are joined using

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T4 DNA ligase. This synthetic minigene, encoding the CTL epitope polypeptide, can then
cloned into a desired expression vector.

Standard regulatory sequences well known to those of skill in the art are
included in the vector to ensure expression in the target cells. Several vector elements are
5   required: a promoter with a down-stream cloning site for minigene insertion; a
polyadenylation signal for efficient transcription termination; an *E. coli* origin of
replication; and an *E. coli* selectable marker (e.g. ampicillin or kanamycin resistance).
Numerous promoters can be used for this purpose, *e.g.*, the human cytomegalovirus
(hCMV) promoter. *See*, U.S. Patent Nos. 5,580,859 and 5,589,466 for other suitable
10   promoter sequences.

Additional vector modifications may be desired to optimize minigene
expression and immunogenicity. In some cases, introns are required for efficient gene
expression, and one or more synthetic or naturally-occurring introns could be incorporated
into the transcribed region of the minigene. The inclusion of mRNA stabilization
15   sequences can also be considered for increasing minigene expression. It has recently been
proposed that immunostimulatory sequences (ISSs or CpGs) play a role in the
immunogenicity of DNA vaccines. These sequences could be included in the vector,
outside the minigene coding sequence, if found to enhance immunogenicity.

In some embodiments, a bicistronic expression vector, to allow production of
20   the minigene-encoded epitopes and a second protein included to enhance or decrease
immunogenicity can be used. Examples of proteins or polypeptides that could beneficially
enhance the immune response if co-expressed include cytokines (e.g., IL2, IL12, GM-
CSF), cytokine-inducing molecules (e.g. LeIF) or costimulatory molecules. Helper (HTL)
epitopes could be joined to intracellular targeting signals and expressed separately from the
25   CTL epitopes. This would allow direction of the HTL epitopes to a cell compartment
different than the CTL epitopes. If required, this could facilitate more efficient entry of
HTL epitopes into the MHC class II pathway, thereby improving CTL induction. In
contrast to CTL induction, specifically decreasing the immune response by co-expression
of immunosuppressive molecules (e.g. TGF-β) may be beneficial in certain diseases.

30   Once an expression vector is selected, the minigene is cloned into the
polylinker region downstream of the promoter. This plasmid is transformed into an
appropriate *E. coli* strain, and DNA is prepared using standard techniques. The

orientation and DNA sequence of the minigene, as well as all other elements included in the vector, are confirmed using restriction mapping and DNA sequence analysis. Bacterial cells harboring the correct plasmid can be stored as a master cell bank and a working cell bank.

5   Therapeutic quantities of plasmid DNA are produced by fermentation in *E. coli*, followed by purification. Aliquots from the working cell bank are used to inoculate fermentation medium (such as Terrific Broth), and grown to saturation in shaker flasks or a bioreactor according to well known techniques. Plasmid DNA can be purified using standard bioseparation technologies such as solid phase anion-exchange resins supplied by 10 Quiagen. If required, supercoiled DNA can be isolated from the open circular and linear forms using gel electrophoresis or other methods.

           Purified plasmid DNA can be prepared for injection using a variety of formulations. The simplest of these is reconstitution of lyophilized DNA in sterile phosphate-buffer saline (PBS). This approach, known as "naked DNA," is currently being 15 used for intramuscular (IM) administration in clinical trials. To maximize the immunotherapeutic effects of minigene DNA vaccines, an alternative method for formulating purified plasmid DNA may be desirable. A variety of methods have been described, and new techniques may become available. Cationic lipids can also be used in the formulation (*see, e.g.,* as described by Debs and Zhu (1993) WO 93/24640; Mannino 20 and Gould-Fogerite (1988) *BioTechniques* 6(7): 682-691; Rose U.S. Pat No. 5,279,833; Brigham (1991) WO 91/06309; and Felgner *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84: 7413-7414). In addition, glycolipids, fusogenic liposomes, peptides and compounds referred to collectively as protective, interactive, non-condensing (PINC) could also be complexed to purified plasmid DNA to influence variables such as stability, intramuscular 25 dispersion, or trafficking to specific organs or cell types.

           The nucleic acids can also be administered using ballistic delivery as described, for instance, in U.S. Patent No. 5,204,253. Particles comprised solely of DNA can be administered. Alternatively, DNA can be adhered to particles, such as gold particles.

30   Target cell sensitization can be used as a functional assay for expression and MHC class 1 presentation of minigene-encoded CTL epitopes. The plasmid DNA is introduced into a mammalian cell line that is suitable as a target for standard CTL

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chromium release assays. The transfection method used will be dependent on the final

formulation. Electroporation can be used for "naked" DNA, whereas cationic lipids allow

direct *in vitro* transfection. A plasmid expressing green fluorescent protein (GFP) can be

co-transfected to allow enrichment of transfected cells using fluorescence activated cell

5   sorting (FACS). These cells are then chromium-51 labeled and used as target cells for

epitope-specific CTL lines. Cytolysis, detected by 51Cr release, indicates production of

MHC presentation of minigene-encoded CTL epitopes.

In vivo immunogenicity is a second approach for functional testing of minigene

DNA formulations. Transgenic mice expressing appropriate human MHC molecules are

10   immunized with the DNA product. The dose and route of administration are formulation

dependent (e.g. IM for DNA in PBS, IP for lipid-complexed DNA). Twenty-one days

after immunization, splenocytes are harvested and restimulated for 1 week in the presence

of peptides encoding each epitope being tested. These effector cells (CTLs) are assayed

for cytolysis of peptide-loaded, chromium-51 labeled target cells using standard

15   techniques. Lysis of target cells sensitized by MHC loading of peptides corresponding to

minigene-encoded epitopes demonstrates DNA vaccine function for *in vivo* induction of

CTLs.

Antigenic peptides may be used to elicit CTL ex vivo, as well. The resulting

CTL, can be used to treat chronic infections (viral or bacterial) or tumors in patients that

20   do not respond to other conventional forms of therapy, or will not respond to a peptide

vaccine approach of therapy. Ex vivo CTL responses to a particular pathogen (infectious

agent or tumor antigen) are induced by incubating in tissue culture the patient's CTL

precursor cells (CTLp) together with a source of antigen-presenting cells (APC) and the

appropriate immunogenic peptide. After an appropriate incubation time (typically 1-4

25   weeks), in which the CTLp are activated and mature and expand into effector CTL, the

cells are infused back into the patient, where they will destroy their specific target cell (an

infected cell or a tumor cell). In order to optimize the *in vitro* conditions for the

generation of specific cytotoxic T cells, the culture of stimulator cells is maintained in an

appropriate serum-free medium.

30   Prior to incubation of the stimulator cells with the cells to be activated, e.g.,

precursor CD8+ cells, an amount of antigenic peptide is added to the stimulator cell

culture, of sufficient quantity to become loaded onto the human Class I molecules to be

expressed on the surface of the stimulator cells. In the present invention, a sufficient amount of peptide is an amount that will allow about 200, and preferably 200 or more, human Class I MHC molecules loaded with peptide to be expressed on the surface of each stimulator cell. Preferably, the stimulator cells are incubated with $>20\mu g/ml$ peptide.

Resting or precursor CD8+ cells are then incubated in culture with the appropriate stimulator cells for a time period sufficient to activate the CD8+ cells. Preferably, the CD8+ cells are activated in an antigen-specific manner. The ratio of resting or precursor CD8+ (effector) cells to stimulator cells may vary from individual to individual and may further depend upon variables such as the amenability of an individual's lymphocytes to culturing conditions and the nature and severity of the disease condition or other condition for which the within-described treatment modality is used. Preferably, however, the lymphocyte:stimulator cell ratio is in the range of about 30:1 to 300:1. The effector/stimulator culture may be maintained for as long a time as is necessary to stimulate a therapeutically useable or effective number of CD8+ cells.

The induction of CTL *in vitro* requires the specific recognition of peptides that are bound to allele specific MHC class 1 molecules on APC. The number of specific MHC/peptide complexes per APC is crucial for the stimulation of CTL, particularly in primary immune responses. While small amounts of peptide/MHC complexes per cell are sufficient to render a cell susceptible to lysis by CTL, or to stimulate a secondary CTL response, the successful activation of a CTL precursor (pCTL) during primary response requires a significantly higher number of MHC/peptide complexes. Peptide loading of empty major histocompatability complex molecules on cells allows the induction of primary cytotoxic T lymphocyte responses. Peptide loading of empty major histocompatability complex molecules on cells enables the induction of primary cytotoxic T lymphocyte responses.

Since mutant cell lines do not exist for every human MHC allele, it is advantageous to use a technique to remove endogenous MHC-associated peptides from the surface of APC, followed by loading the resulting empty MHC molecules with the immunogenic peptides of interest. The use of non-transformed (non-tumorigenic), non-infected cells, and preferably, autologous cells of patients as APC is desirable for the design of CTL induction protocols directed towards development of *ex vivo* CTL therapies. This application discloses methods for stripping the endogenous MHC-associated peptides from the surface of APC followed by the loading of desired peptides.

A stable MHC class 1 molecule is a trimeric complex formed of the following elements: 1) a peptide usually of 8 - 10 residues, 2) a transmembrane heavy polymorphic protein chain which bears the peptide-binding site in its α1 and α2 domains, and 3) a

non-covalently associated non-polymorphic light chain, $\beta_2$microglobulin. Removing the bound peptides and/or dissociating the $\beta_2$microglobulin from the complex renders the MHC class I molecules nonfunctional and unstable, resulting in rapid degradation. All MHC class I molecules isolated from PBMCs have endogenous peptides bound to them.

5   Therefore, the first step is to remove all endogenous peptides bound to MHC class I molecules on the APC without causing their degradation before exogenous peptides can be added to them.

Two possible ways to free up MHC class I molecules of bound peptides include lowering the culture temperature from 37°C to 26°C overnight to destablize

10   $\beta_2$microglobulin and stripping the endogenous peptides from the cell using a mild acid treatment. The methods release previously bound peptides into the extracellular environment allowing new exogenous peptides to bind to the empty class I molecules. The cold-temperature incubation method enables exogenous peptides to bind efficiently to the MHC complex, but requires an overnight incubation at 26°C which may slow the cell's

15   metabolic rate. It is also likely that cells not actively synthesizing MHC molecules (e.g., resting PBMC) would not produce high amounts of empty surface MHC molecules by the cold temperature procedure.

Harsh acid stripping involves extraction of the peptides with trifluoroacetic acid, pH 2, or acid denaturation of the immunoaffinity purified class I-peptide complexes.

20   These methods are not feasible for CTL induction, since it is important to remove the endogenous peptides while preserving APC viability and an optimal metabolic state which is critical for antigen presentation. Mild acid solutions of pH 3 such as glycine or citrate-phosphate buffers have been used to identify endogenous peptides and to identify tumor associated T cell epitopes. The treatment is especially effective, in that only the

25   MHC class I molecules are destabilized (and associated peptides released), while other surface antigens remain intact, including MHC class II molecules. Most importantly, treatment of cells with the mild acid solutions do not affect the cell's viability or metabolic state. The mild acid treatment is rapid since the stripping of the endogenous peptides occurs in two minutes at 4°C and the APC is ready to perform its function after the

30   appropriate peptides are loaded. The technique is utilized herein to make peptide-specific APCs for the generation of primary antigen-specific CTL. The resulting APC are efficient in inducing peptide-specific CD8+ CTL.

Activated CD8+ cells may be effectively separated from the stimulator cells using one of a variety of known methods. For example, monoclonal antibodies specific for the stimulator cells, for the peptides loaded onto the stimulator cells, or for the CD8+ cells (or a segment thereof) may be utilized to bind their appropriate complementary ligand. Antibody-tagged molecules may then be extracted from the stimulator-effector cell admixture via appropriate means, e.g., via well-known immunoprecipitation or immunoassay methods.

Effective, cytotoxic amounts of the activated CD8+ cells can vary between *in vitro* and *in vivo* uses, as well as with the amount and type of cells that are the ultimate target of these killer cells. The amount will also vary depending on the condition of the patient and should be determined via consideration of all appropriate factors by the practitioner. Preferably, however, about 1×10^6 to about 1×10^{12}, more preferably about 1×10^8 to about 1×10^{11}, and even more preferably, about 1×10^9 to about 1×10^{10} activated CD8+ cells are utilized for adult humans, compared to about $5 \times 10^6 - 5 \times 10^7$ cells used in mice.

Preferably, as discussed above, the activated CD8+ cells are harvested from the cell culture prior to administration of the CD8+ cells to the individual being treated. It is important to note, however, that unlike other present and proposed treatment modalities, the present method uses a cell culture system that is not tumorigenic. Therefore, if complete separation of stimulator cells and activated CD8+ cells is not achieved, there is no inherent danger known to be associated with the administration of a small number of stimulator cells, whereas administration of mammalian tumor-promoting cells may be extremely hazardous.

Methods of re-introducing cellular components are known in the art and include procedures such as those exemplified in U.S. Patent No. 4,844,893 to Honsik, et al. and U.S. Patent No. 4,690,915 to Rosenberg. For example, administration of activated CD8+ cells via intravenous infusion is appropriate.

The immunogenic peptides of this invention may also be used to make monoclonal antibodies. Such antibodies may be useful as potential diagnostic or therapeutic agents.

The peptides may also find use as diagnostic

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reagents. For example, a peptide of the invention may be used to determine the susceptibility of a particular individual to a treatment regimen which employs the peptide or related peptides, and thus may be helpful in modifying an existing treatment protocol or in determining a prognosis for an affected individual. In addition, the peptides may also

5   be used to predict which individuals will be at substantial risk for developing chronic infection.

To identify peptides of the invention, class I antigen isolation, and isolation and sequencing of naturally processed peptides was carried out as described in the related applications. These peptides were then used to define specific binding motifs for each of

10   the following alleles A3.2, A1, A11, and A24.1. These motifs are described in the related applications and summarized in Tables 5-8, below.

TABLE 5
Summary
HLA-A3.2 Allele-Specific Motif

Position	Conserved Residues
1	-
2	V,L,M
3	Y,D
4	-
5	-
6	-
7	I
8	Q,N
9	K
10	K

TABLE 6
Summary
HLA-A1 Allele-Specific Motif

Position	Conserved Residues
1	-
2	S, T
3	D,E
4	P
5	-
6	-
7	L
8	-
9	Y

TABLE 7
Summary
HLA-A11 Allele-Specific Motif

Position	Conserved Residues
1	-
2	T,V
3	M,F
4	-
5	-
6	-
7	-
8	Q
9	K
10	K

Table 8
Summary
HLA-A24.1 Allele-Specific Motif

Position	Conserved Residues
1	-
2	Y
3	I,M
4	D,E,G,K,P
5	L,M,N
6	V
7	N,V
8	A,E,K,Q,S
9	F,L
10	F,A

Example 1

Identification of immunogenic peptides

Using the motifs identified above for various MHC class I allele amino acid sequences from various viral and tumor-related proteins were analyzed for the presence of

5   these motifs. Screening was carried out described in the related applications. Table 9 provides the results of searches of the antigens.

The above description is provided to illustrate the invention but not to limit its scope. Other variants of the invention will be readily apparent to one of ordinary skill in the art and are encompassed by the appended claims. All publications, patents, and patent

10   applications cited herein are hereby incorporated by reference.

Table 9

SEQ. ID. No.	AA	SEQUENCE	SOURCE	ORIGIN_OR MOTIF
1	9	HSNLNDTTY	FLU A NP 140	A03
2	9	FVEALFQEY	P. falciparum CSP	A03/A11
3	9	AADAAAAAY	A1 poly-A	A01
4	9	DADAAAAY	A1 poly-A	A01
5	9	AADSAAAAY	A1 poly-A	A01
6	9	AADAPAAAY	A1 poly-A	A01
7	9	AADAAAQAY	A1 poly-A	A01
8	9	QADAAAAAY	A1 poly-A	A01
9	9	AADADAAAY	A1 poly-A	A01
10	9	AADAGAAAY	A1 poly-A	A01
11	9	AADAAKAAY	A1 poly-A	A01
12	9	AADAAAPAY	A1 poly-A	A01
13	9	AADAAAAPY	A1 poly-A	A01
14	9	ATAAAAAAY	A1 poly-A	A01
15	9	DTAAAAAAY	A1 poly-A	A01
16	9	ATASAAAAY	A1 poly-A	A01
17	9	ATAAPAAAY	A1 poly-A	A01
18	9	ATAAAQAY	A1 poly-A	A01
19	9	ATAAAAAKY	A1 poly-A	A01
20	9	QTAAAAAAY	A1 poly-A	A01
21	9	ATAADAAAY	A1 poly-A	A01
22	9	ATAAGAAAY	A1 poly-A	A01
23	9	ATAAAKAAY	A1 poly-A	A01
24	9	ATAAAAPAY	A1 poly-A	A01
25	9	ATAAAAAPY	A1 poly-A	A01
26	9	TYLISSIPL	GCDFP-15 70	A24
27	9	FYTNRTVQI	GCDFP-15 102	A24
28	10	PLQGAFNYKY	GCDFP-15 77	A01
29	10	LCDDNPKTFY	GCDFP-15 90	A01
30	9	TTNLRPTTY	GAD 21	A01
31	9	CLELAEYLY	GAD 483	A01
321	9	LLSPRPISY	HCV NS3 1157	A01
33	9	LLSPRPVSY	HCV NS3 1157	A01
34	9	PTVTVFHVY	HSV-1 POL 142	A01
35	9	ETAGRHVGY	HSV-1 POL 688	A01
36	9	GSGPELLFY	HSV-1 TERM 612	A01

SEQ. ID. No.	AA	SEQUENCE	SOURCE	ORIGIN_OR MOTIF
37	9	LSPQWVADY	HSV-1 ENV 143	A01
38	9	VVERTDVYY	HSV-1 POL 252	A01
39	9	SLEHTLCTY	HSV-1 ENV 587	A01
40	9	PSQRHGSKY	Hu MBP 6	A01
41	9	CSAVPVYIY	Hu PLP 169	A01
42	9	LTFMIAATY	Hu PLP 255	A01
43	9	GTASFFFLY	Hu PLP 74	A01
44	9	GTEKLIETY	Hu PLP 42	A01
45	9	TTWCSQTSY	LCMV GP 217	A01
46	9	RTWENHCTY	LCMV GP 233	A01
47	9	QSSINISGY	LCMV NUC 232	A01
48	9	ITEMLRKDY	LCMV GP 417	A01
49	9	QSSFYSDWY	M. Tuberc. 85A/3	A01
50	9	SSALTLAIY	M. Tuberc. 85A/3	A01
51	9	ATWLGDDGY	M. Tuberc. cat/p	A01
52	9	QSTSINLPY	M. Tuberc. DNAK	A01
53	9	QSSFYSDWY	M. Tuberc. 75	A01
54	9	YAELMTADY	M. Tuberc. POL	A01
55	9	STNEVTRIY	PSM 348	A01
56	9	RVDCTPLMY	PSM 463	A01
57	9	RGRRQPIPK	HCV CORE 59	A03/A11
58	9	KTKRNTNRR	HCV CORE 10	A03/A11
59	9	LGFGAYMSK	HCV NS3 1267	A03/A11
60	9	VAGALVAFK	HCV NS4 1864	A03/A11
61	9	NFISGIQYL	HCV NS4 1772	A24
62	9	FWAKHMWNF	HCV NS4 1765	A24
63	10	EVDGVRLHRY	HCV NS5 2129	A01
64	10	DLSGWFVAGY	HCV NS5 2999	A01
65	10	AACNWTRGER	HCV NS1/E2 647	A03/A11
66	9	KVYLAWVPA	HIV-1 POL 74	A03
67	9	TLFCASDAK	HIV-1 ENV 82	A03/A11
68	9	ISLWDQSLK	HIV-1 ENV 78	A03/A11
69	9	RIVELLGRR	HIV-1 ENV 53	A03/A11
70	9	MVHQAISPR	HIV-1 GAG 45	A03/A11
71	9	TIKIGGQLK	HIV-1 POL 65	A03/A11
72	9	KLVSAGIRK	HIV-1 POL 57	A03/A11
73	9	KGLGISYGR	HIV-1 TAT 77	A03/A11

SEQ. ID. No.	AA	SEQUENCE	SOURCE	ORIGIN_OR MOTIF
74	9	GLGISYGRK	HIV-1 TAT 77	A03/A11
75	9	VMIVWQVDR	HIV-1 VIF 83	A03/A11
76	9	QMAVFIHNF	HIV-1 POL 92	A03/A24
77	9	SMTKILEPF	HIV-1 POL 87	A03/A24
78	9	IWGCSGKLI	HIV-1 ENV 69	A24
79	9	LYKYKVVKI	HIV-1 ENV 49	A24
80	9	VWKEATTTL	HIV-1 ENV 47	A24
81	9	GWMTNNPPI	HIV-1 GAG 31	A24
82	9	RFAVNPGLL	HIV-1 GAG 26	A24
83	9	PYNTPVFAI	HIV-1 POL 74	A24
84	9	WWAGIKQEF	HIV-1 POL 70	A24
85	9	LWQRPLVTI	HIV-1 POL 61	A24
86	9	IYETYGDTW	HIV-1 VPR 92	A24
87	9	PYNEWTLEL	HIV-1 VPR 56	A24
88	10	ILQQLLFIHF	HIV-1 VPR 72	A03
89	10	TTLFCASDAK	HIV-1 ENV 81	A03/A11
90	10	LLGIWGCSGK	HIV-1 ENV 73	A03/A11
91	10	IISLWDQSLK	HIV-1 ENV 66	A03/A11
92	10	LLQLTVWGIK	HIV-1 ENV 61	A03/A11
93	10	SILDIRQGPK	HIV-1 GAG 72	A03/A11
94	10	QMVHQAISPR	HIV-1 GAG 45	A03/A11
95	10	TAVQMAVFIH	HIV-1 POL 88	A03/A11
96	10	ISPIETVPVK	HIV-1 POL 87	A03/A11
97	10	LGIPHPAGLK	HIV-1 POL 87	A03/A11
98	10	PAIFQSSMTK	HIV-1 POL 78	A03/A11
99	10	KVYLAWVPAH	HIV-1 POL 74	A03/A11
100	10	DIIATDIQTK	HIV-1 POL 67	A03/A11
101	10	VTIKIGGQLK	HIV-1 POL 65	A03/A11
102	10	KAACWWAGIK	HIV-1 POL 65	A03/A11
103	10	VSQIIEQLIK	HIV-1 POL 61	A03/A11
104	10	KGLGISYGRK	HIV-1 TAT 77	A03/A11
105	10	VWKEATTTLF	HIV-1 ENV 47	A24
106	10	YWQATWIPEW	HIV-1 POL 96	A24
107	10	VYYDPSKDLI	HIV-1 POL 70	A24
108		ALAAGAAAR	A3 poly-A	A03
109		AAAAGAAAK	A3 poly-A	A03
110	9	AFLPWHRLF	Tyrosinase	A24

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SEQ. ID. No.	AA	SEQUENCE	SOURCE	ORIGIN__OR MOTIF
111	9	AYGLDFYIL	p15	A24

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WHAT IS CLAIMED IS:

1.   A method of inducing a cytotoxic T cell response against a preselected antigen in a patient, the method comprising contacting cytotoxic T cells from the patient with an immunogenic peptide which binds to an HLA-A3.2 MHC product with a dissociation constant of less than about 5×10^{-7} M and induces a cytotoxic T cell response, which immunogenic peptide has between about 9 and about 10 residues and the following residues, from the N-terminus to the C-terminus:

a first conserved residue selected from the group consisting of L, M, I, V, S, A, T, F, C, G, D and E;

and a second conserved residue of K, R, Y, H and F;

wherein the first and second conserved residues are separated by 6 to 7 residues.

2.   The method of claim 1, wherein the first conserved residue is at the second position from the N-terminus.

3.   A method of inducing a cytotoxic T cell response against a preselected antigen in a patient, the method comprising contacting cytotoxic T cells from the patient with an immunogenic peptide which binds to an HLA-A1 MHC product with a dissociation constant of less than about 5×10^{-7} M and induces a cytotoxic T cell response, which immunogenic peptide has between about 9 and about 10 residues and the following residues, from the N-terminus to the C-terminus:

a first conserved residue of T, S and M; and

a second conserved residue of D, E, A, S and T;

a third conserved residue of Y;

wherein the first and second conserved residues are adjacent and the second and third conserved residues are separated by 5 or 6 residues.

4.   The method of claim 3, wherein the first conserved residue is at the second position from the N-terminus.

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5.   A method of inducing a cytotoxic T cell response against a preselected antigen in a patient, the method comprising contacting cytotoxic T cells from the patient with an immunogenic peptide which binds to an HLA-A1 MHC product with a dissociation constant of less than about 5×10^{-7} M and induces a cytotoxic T cell response, which immunogenic peptide has between about 9 and about 10 residues and the following residues, from the N-terminus to the C-terminus:

        a first conserved residue of T, S and M; and

        a second conserved residue of Y;

        wherein the first and second conserved residues are separated by 6 to 7 residues.

6.   The method of claim 5, wherein the first conserved residue is at the second position from the N-terminus and the second conserved residue is at the ninth or tenth position from the N-terminus.

7.   A method of inducing a cytotoxic T cell response against a preselected antigen in a patient, the method comprising contacting cytotoxic T cells from the patient with an immunogenic peptide which binds to an HLA-A1 MHC product with a dissociation constant of less than about 5×10^{-7} M and induces a cytotoxic T cell response, which immunogenic peptide has between about 9 and about 10 residues and the following residues, from the N-terminus to the C-terminus:

        a first conserved residue of D, E, A, S and T; and

        a second conserved residue of Y;

        wherein the first and second conserved residues are separated by 5 to 6 residues.

8.   The method of claim 7, wherein the first conserved residue is at the third position from the N-terminus and the second conserved residue is at the ninth or tenth position from the N-terminus.

9.   A method of inducing a cytotoxic T cell response against a preselected antigen in a patient, the method comprising contacting cytotoxic T cells from the patient

with an immunogenic peptide which binds to an HLA-A11 MHC product with a
dissociation constant of less than about 5 x 10^{-7} M and induces a cytotoxic T cell response,
which immunogenic peptide has between about 9 and about 10 residues and the following
residues, from the N-terminus to the C-terminus:

    a first conserved residue of L, M, I, V, A, S, T, G, N, Q, C, F, D, E; and

    a second conserved residue of K, R, H;

    wherein the first and second conserved residues are separated by 6 to 7
residues.

10.   The method of claim 9, wherein the first conserved residue is at the
second position from the N-terminus.

11.   A method of inducing a cytotoxic T cell response against a preselected
antigen in a patient, the method comprising contacting cytotoxic T cells from the patient
with an immunogenic peptide which binds to an HLA-A24.1 MHC product with a
dissociation constant of less than about 5 x 10^{-7} M and induces a cytotoxic T cell response,
which immunogenic peptide has between about 9 and about 10 residues and the following
residues, from the N-terminus to the C-terminus:

    a first conserved residue of Y, F, W; and

    a second conserved residue of F, I, L, W, M;

    wherein the first and second conserved residues are separated by 6 to 7
residues.

12.   The method of claim 11, wherein the first conserved residue is at the
second position from the N-terminus.

13.   A method of inducing a cytotoxic T cell response against a preselected
antigen in a patient, the method comprising contacting cytotoxic T cells from the patient
with an immunogenic peptide which binds to an HLA-A3.2 MHC product with a
dissociation constant of less than about 5 x 10^{-7} M and induces a cytotoxic T cell response,
which immunogenic peptide has between about 9 and about 10 residues and the following
residues, from the N-terminus to the C-terminus:

a first conserved residue at the second position selected from the group consisting of A, I, L, M, T, and V; and a second conserved residue at the C terminal position selected from the group consisting of K and R.

wherein the first and second conserved residues are separated by 6 to 7 residues.

14. A method of inducing a cytotoxic T cell response against a preselected antigen in a patient, the method comprising contacting cytotoxic T cells from the patient with an immunogenic peptide which binds to an HLA-A3.2 MHC product with a dissociation constant of less than about 5×10^{7} M and induces a cytotoxic T cell response, which immunogenic peptide has between about 9 and about 10 residues and the following residues, from the N-terminus to the C-terminus:

a first conserved residue at the second position from the N terminus selected from the group consisting of A, I, L, M, T and V; and

a second conserved residue at the C terminal position selected from the group consisting of K;

wherein the first and second conserved residues are separated by 6 to 7 residues.

15. The method of claims 1, 3, 5, 7, 11, 13, or 14, wherein the immunogenic peptide is contacted with the cytotoxic T cell *in vitro*.

16. The method of claims 1, 3, 5, 7, 11, 13, or 14, wherein the step of contacting cytotoxic T cells with the immunogenic peptide is carried out by administering to the patient a nucleic acid encoding the peptide.

17. The method of claims 1, 3, 5, 7, 11, 13, or 14, 5, wherein the immunogenic peptide is from a viral antigen.

18. The method of claims 1, 3, 5, 7, 11, 13, or 14, wherein the immunogenic peptide is from a cancer antigen.

19.   A composition comprising an immunogenic peptide, wherein the immunogenic peptide is selected from the group consisting of SEQ. ID. Nos. 1-111.

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A.	CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/00, 38/08; CO7K 7/00
US CL   :424/185.1; 530/300, 350, 395, 868
According to International Patent Classification (IPC) or to both national classification and IPC

B.	FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/185.1; 530/300, 350, 395, 868

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
EXAMINER'S CELLULAR IMMUNOLOGY REFERENCE FILE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
AUTOMATED PATENT SYSTEM (APS) AND DIALOG (FILE=BIOCHEM) DATABASES: AGRETOPE, MAJOR
HISTOCOMPAT?, HLA-A1?, HLA-A3?, HLA-A11?, HLA-A24?

C.	DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ENGELHARD, V.H. Structure of peptides associated with MHC Class I molecules, Curr. Opin. Immunology, 1994, volume 6, pages 13-23, see entire document.	1-19
Y	PARKER, K.C. et al. Sequence motifs important for peptide binding to the human MHC Class I molecule, HLA-A2, J. Immunology, 11 December 1992, Volume 149, pages 3580-3587, see abstract.	1-19
Y	RAMMENSEE, H. et al. Peptides naturally presented by MHC Class I molecules. Annu. Rev. Immunol. 1993, Volume 11, pages 213-244, see entire article.	1-19

[X]	Further documents are listed in the continuation of Box C.	[ ]	See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)		
"O"	document referring to an oral disclosure, use, exhibition or other means	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 APRIL 1997	1 1 JUL 1997

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CELIS, E. et al. Identification of potential CTL epitopes of tumor-associated antigen MAGE-1 for five common HLA-A alleles, Mol. Immunology, Volume 31, No. 8, December 1994, pages 1423-1430, see entire article.	1-19